

RECEIVED

O I P Inventor: Baback Gharizadeh
Address: Högalidsgatan 33, 1 tr, SE-11730, Sweden Tel work: +46-8-5537 8393 home: +46-8-34 82 97

JAN 08 2004



TECH CENTER 1600/2900

My claims for the invention are:

- 1 The multiple sequencing oligonucleotide primer pool method is utilized for specific genotyping, typing, identification, detection and sequencing of a sample of nucleic acid molecules, whereby the molecules are suspected to contain at least one type or species or target of a variable region, region of interest or target DNA, each type having different nucleotide patterns, and the molecules are suspected to contain unspecific amplification in the amplification product and comprising the steps of:
 - A. Providing the sample is of nucleic acid molecules;
 - B. Providing a mixed set of at least two sequencing oligonucleotide primers, whereby each primer is designed for being specific for one type or species or group or target chosen from the known set of types or target of the nucleic acid sample, thereby allowing a primer, which is specific for a type, species, group or target that is present in the sample, to hybridize in or close to the target or variable region;
 - C. Mixing the set of sample and specific primers under conditions allowing a primer or primers to hybridize if a target type or types are present in the sample;
 - D. Determining the type, species or target region to which the primer or primers have hybridized by extending the hybridized primer or primers in a DNA sequencing reaction.
- 2 Method according to claim 1, wherein the sequencing reaction is performed by sequencing-by-synthesis, Sanger dideoxy sequencing, sequencing by mass spectrometry or any other DNA sequencing technology, applicable to the method of invention.
- 3 Method according to any one of claim 1-2, wherein the sample is a microorganism, such as a virus, fungi or bacteria.
- 4 Method according to any one of claim 1-3, wherein the sample is suspected to comprise at least two types, species or targets of nucleic acid molecules chosen from the known set of types, species or targets.
- 5 Method according to claim 4, wherein the sample contains multiple infection or variants or types or species.

- 6 Method according to claim 5, wherein at least one primer is specific for a variant of a disease linked to the microorganism.
- 7 Method according to claim 6, wherein the microorganism is a human papillomavirus
- 8 Method according to claim 7, wherein the known set of HPV-types are chosen from the group comprising the HPV-types; high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, 69 and low-risk: 6, 11, 34, 40, 42, 43, 44
- 9 Method according to any one of the preceding claims, wherein the sequence distance from the 3'-end of the general or consensus extendable primer could be avoided and to be instead sequenced in semi-conservative regions
- 10 Method according to any one of the preceding claims, for typing of amplicons comprising at least one semi-conservative region.
- 11 Method according to any one of claim 1-10 for typing of samples where a type or species is in minority or if the amplification fragment has low yield.
- 12 Method according to any one of claim 1-11, for typing of samples by DNA sequencing that contain unspecific amplification products, the primers of the set of primers not annealing to unspecific amplification products.
- 13 Kit for use in the method of typing of claim 1-12, comprising at least two oligonucleotide primers, whereby each primer is designed for being specific for one genotype or type or species or group or target or type-specific region chosen from a known set of types of the type-specific or target region of a nucleic acid sample, thereby allowing a primer, which is specific for a genotype or type or species or target that is present in the sample, to hybridize in or close to the type-specific or target region.
- 14 Kit according to claim 13, wherein the oligonucleotide primers of the kit are designed for being specific for any of the HPV-types chosen from the group comprising: high-risk: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66, 68, 69 and low-risk: HPV-6, 11, 34, 40, 42, 43, 44
- 15 Kit according to any one of the preceding claims for genotyping, typing, detection, identification or sequencing of any microorganisms, viruses or any other application where the multiple sequencing oligonucleotide primer pool approach is applicable by DNA sequencing technologies

Inventor: Baback Gharizadeh

Address: Högalidsgatan 33, 1 tr, SE-11730, Sweden Tel work: +46-8-5537 8393 home: +46-8-34 82 97

16 Kit according to claim 1 for quantitative measurements of different genotypes or species or types amplified by PCR in the same sample or by mixing different amplicons